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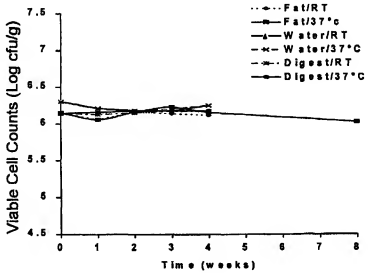
(54) Cereal product containing probiotics

(57) A dried, ready-to-eat cereal product comprising a gelatinised starch matrix which includes a coating or filling which contains a probiotic micro-organism. The cereal product may be in the form of a pet food, a breakfast cereal, an infant cereal or a convenience food. The cereal product may be produced by cooking a starch source to form a gelatinised starch matrix; forming the gelatinised matrix into pieces; drying the pieces; and coating or filling the pieces with a carrier which contains probiotic micro-organisms.

FIG.1.



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Description

Field of the Invention

This invention relates to a ready-to-eat cereal product which contains a probiotic micro-organism; for example pet foods, breakfast cereals, infant cereals or convenience foods. In use, the cereal product has a beneficial effect in the gastro-intestinal tract of the person or animal consuming it and hence upon the person or animal. The invention also relates to a process of producing the cereal product and to methods of promoting beneficial effects in the gastro-intestinal tracts of humans and animals.

Background of the Invention

Probiotic micro-organisms are micro-organisms which beneficially affect a host by improving its intestinal microbial balance (Fuller, R; 1989; J. Applied Bacteriology, 66: 365-378). In general, probiotic micro-organisms produce organic acids such as lactic acid and acetic acid which inhibit the growth of pathogenic bacteria such as *Clostridium perfringens* and *Helicobacter pylori*. Consequently, probiotic bacteria are believed to be useful in the treatment and prevention of conditions caused by pathogenic bacteria. Further, probiotic micro-organisms are believed to inhibit the growth and activity of putrefying bacteria and hence the production of toxic amine compounds. It is also believed that probiotic bacteria activate the immune function of the host.

Therefore there is considerable interest in including probiotic micro-organisms into foodstuffs. For example, many fermented milk products which contain probiotic micro-organisms are commercially available. Usually these products are in the form of yoghurts and an example is the LC1® yoghurt (Société des Produits Nestlé SA). Several infant and follow-up formulas which contain probiotic micro-organisms are also commercially available; for example the BIO NAN® formula (Société des Produits Nestlé SA).

Similarly, for animals, there has been interest in including probiotic micro-organisms into animal feeds. For example, Russian patent 2018313 discloses a powdered, spray-dried animal feed which is based upon milk and which contains certain bifidobacteria and streptococci. The animal feed is aimed primarily at live stock although it is mentioned that the feed may be fed to pets.

However, there are two main issues in incorporating probiotic micro-organisms into foodstuffs. First, the foodstuff must be in a form which is palatable to a consumer. Secondly, the probiotic micro-organism must remain viable during storage. The second issue is particularly problematic for ready-to-eat cereal products. These cereal products, unlike fermented milks, are required to have long storage lives; for example at least a year while the cell counts for many probiotic micro-organisms may fall away completely within one or two days. This is particularly the case if the water activity of the foodstuff is above about 0.5. This is usually the case for dried pet foods.

Therefore there is a need for a ready-to-eat cereal product which contains a probiotic micro-organism, is highly palatable, and which is storage stable.

Summary of the Invention

Accordingly, in one aspect, this invention provides a dried, ready-to-eat cereal product comprising a gelatinised starch matrix which includes a coating or filling containing a probiotic micro-organism.

It has been found that probiotic micro-organisms remain viable for extended periods of time when formulated into a coating on or filling in a dried cereal product. This is surprising since probiotic micro-organisms ordinarily die off rapidly. This is particularly the case for dried, cooked pet foods which generally have a water activity of above about 0.5; levels at which probiotic micro-organisms ordinarily die off rapidly. Therefore the invention offers the advantage of a ready-to-eat cereal product which is highly palatable and which contains a shelf stable source of probiotic micro-organisms.

The cereal product may be in the form of a dried pet food, breakfast cereal, an infant cereal, or a convenience food such as a cereal bar. For human foods, the gelatinised starch matrix is preferably in flaked or expanded form. For pet foods, the gelatinised starch matrix is preferably in the form of pieces or pellets. The gelatinised matrix is preferably produced by extrusion cooking a starch source.

Preferably the coating comprises a carrier substrate which carries the probiotic micro-organism in it. The filling may also comprise a carrier substrate which carries the probiotic micro-organism in it. For example, the carrier substrate may be protein digest, fat, milk solids, sugar or a particulate flavouring agent.

In a further aspect, this invention provides a process of preparing a dried, ready-to-eat cereal product, the process comprising cooking a starch source to form a gelatinised starch matrix; forming the gelatinised starch matrix into pieces and drying the pieces; and coating or filling the pieces with a substrate which contains probiotic micro-organisms.

In one embodiment, the gelatinised starch matrix is formed into pieces and dried by extruding the gelatinised matrix

to form a cooked extrudate and cutting and drying the cooked extrudate to form dried pieces. The gelatinised matrix may be caused to expand upon extrusion to form, after cutting and drying, expanded pieces. Alternatively, the pieces may be subjected to flaking to form flaked pieces.

In another embodiment, the gelatinised starch matrix may be formed into pieces and dried by roller-drying the gelatinised starch matrix to form flakes.

In a further embodiment, the gelatinised starch matrix may be formed into pieces and dried by extruding the gelatinised matrix to form a cooked extrudate containing an aperture; and cutting and drying the pieces. Preferably the gelatinised starch matrix is extruded with a central bore for receiving a filling.

Brief Description of the Drawings

Embodiments of the invention are now described, by way of example only, with reference to the drawings in which:

Figure 1 is a graph illustrating the viability of *Bacillus coagulans* in various coatings on a dried, cooked, pet food; and

Figure 2 is a graph illustrating the viability of *Bacillus subtilis* in various coatings on a dried, cooked, pet food.

Detailed description of preferred embodiments of the invention

Embodiments of the invention are now described, by way of example only. The invention provides a dried, ready-to-eat cereal product in the form of a gelatinised starch matrix which includes a coating or filling. The coating or filling contains a probiotic micro-organism. The probiotic micro-organism may be selected from one or more micro-organisms suitable for human or animal consumption and which is able to improve the microbial balance in the human or animal intestine.

Examples of suitable probiotic micro-organisms include yeasts such as *Saccharomyces*, *Debaromyces*, *Candida*, *Pichia* and *Torulopsis*, moulds such as *Aspergillus*, *Rhizopus*, *Mucor*, and *Penicillium* and *Torulopsis* and bacteria such as the genera *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*, *Melissococcus*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Peptostrepococcus*, *Bacillus*, *Pediococcus*, *Micrococcus*, *Leuconostoc*, *Weissella*, *Aerococcus*, *Oenococcus* and *Lactobacillus*. Specific examples of suitable probiotic micro-organisms are: *Saccharomyces cereviseae*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Enterococcus faecium*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Lactobacillus alimentarius*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus casei Shirota*, *Lactobacillus curvatus*, *Lactobacillus delbruckii* subsp. *lactis*, *Lactobacillus farciminus*, *Lactobacillus gasseri*, *Lactobacillus helveticus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus* (*Lactobacillus* GG), *Lactobacillus sake*, *Lactococcus lactis*, *Micrococcus varians*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Pediococcus halophilus*, *Streptococcus faecalis*, *Streptococcus thermophilus*, *Staphylococcus carnosus*, and *Staphylococcus xylosus*. The probiotic micro-organisms are preferably in powdered, dried form; especially in spore form for micro-organisms which form spores. Further, if desired, the probiotic micro-organism may be encapsulated to further increase the probability of survival; for example in a sugar matrix, fat matrix or polysaccharide matrix.

The dried, ready-to-eat cereal product may be produced from any suitable ingredients; such as those commonly used in dried, ready-to-eat cereal products. One of these ingredients is a starch source. Suitable starch sources are, for example, grain flours such as corn, rice, wheat, beets, barley, soy and oats. Also mixtures of these flours may be used. The flours may be whole flours or may be flours which have had fractions removed; for example the germ fraction or husk fraction may be removed. Rice flour, corn flour and wheat flour are particularly suitable; either alone or in combination. The starch source will be chosen largely on the basis of the nutritional value, palatability considerations, and the type of cereal product desired.

The cereal product may also contain a starch source. Suitable protein sources may be selected from any suitable animal or vegetable protein source; for example meat meal, bone meal, fish meal, soy protein concentrates, milk proteins, gluten, and the like. The choice of the protein source will be largely determined by the nutritional needs, palatability considerations, and the type of cereal product produced. Of course, the starch source may also be a source of protein.

The cereal product may be produced in many different ways as desired. However, an especially suitable way of producing the cereal product is by extrusion cooking. This may be done as is well known in the art. For example, in one suitable process, a feed mixture is fed into a preconditioner. The feed mixture is primarily made up of the starch source and other ingredients such as sugar, salt, spices, seasonings, vitamins, minerals, flavouring agents, colouring agents, antioxidants, protein sources, fats and the like. If desired, sources of insoluble fibre may also be included; for example wheat bran, corn bran, rice bran, rye bran and the like. Further, if desired, a source of soluble fibre may be included, for

example, chicory fibres, inulin, fluctooligosaccharides, soy oligosaccharides, oat bran concentrate, guar gum, carob bean gum, xantham gum, and the like. Preferably the soluble fibre selected is a substrate for the micro-organism selected, or such that the soluble fibre and micro-organism form a symbiotic relationship for promoting beneficial effects. The maximum level of soluble fibre is preferably about 20% by weight; especially about 10% by weight. For example, for pet foods, chicory may be included to comprise about 1% to about 20% by weight of the feed mixture; more preferably about 2% to about 10% by weight.

Depending upon the desired form of the cereal product, the starch content of the feed mixture may be varied. For example, for an expanded cereal product, the feed mixture preferably includes up to about 40% by weight of starch. However, for a flaked product, it is not necessary to use large amounts of starch in the feed mixture since it is possible to flake an unexpanded product.

In the preconditioner, water or steam, or both, is mixed into the feed mixture. Sufficient water or steam is mixed into the feed mixture to moisten the feed mixture. If desired, the temperature of the feed mixture may be raised in the preconditioner to about 60°C to about 90°C by weight. A suitable preconditioner is described in US patent 4,752,139. It is not necessary to subject the feed mixture to preconditioning but it is advantageous to do so.

The moistened feed leaving the preconditioner is then fed into an extruder. The extruder may be any suitable single or twin screw, cooking-extruder. Suitable extruders may be obtained from Wenger Manufacturing Inc, Clextral SA, Bühler AG, and the like. During passage through the extruder, the moistened feed passes through a cooking zone, in which it is subjected to mechanical shear and is heated; for example up to a maximum temperature of up to about 150°C, and a forming zone. The gauge pressure in the forming zone is about 300 kPa to about 10 MPa, as desired. If desired, water or steam, or both, may be introduced into the cooking zone. During passage through the extruder, the starch source of the moistened feed is gelatinised to provide a gelatinised starch matrix.

If desired, a small amount of an edible oil may be fed into the extruder along with the moistened feed to facilitate the extrusion process or as a carrier for oil soluble additives. Any suitable oil may be used; for example vegetable oils such as sunflower oil, safflower oil, corn oil, and the like. If oils are used, oils which are high in mono-unsaturates are particularly preferred. Hydrogenated oils or fats are also preferred. The amount of oil used is preferably kept below about 1% by weight.

The gelatinised matrix leaving the extruder is forced through a suitable die; for example a die as described in European patent application 0665051; the disclosure of which is incorporated by reference. A shaped extrudate, which has a cross-sectional shape corresponding to that of the orifice of the die, leaves the die. If it is desired to produce a centred-filled cereal product, the gelatinised matrix may be extruded with a central bore. The shaped extrudate is then cut into pieces using rotating blades at the exit of the die. Depending upon the conditions in the extruder and the composition of the shaped extrudate, the shaped extrudate expands to a greater or lesser extent. In the case of pet foods, little or no expansion usually takes place.

If a flaked product is to be produced, the pieces may then be transferred to a flaking apparatus. Suitable apparatus are well known and widely used in the cereal industry and may be purchased from, for example, Bühler AG in Switzerland. If desired, the pieces may be partially dried before flaking.

The pieces are then dried to a moisture content below about 10% by weight. This is conveniently carried out in a hot air drier as is conventional. For breakfast cereals, moisture contents of about 1% to about 3% by weight are preferred.

The pieces intended for pet foods may be in the form of chewable pieces. The pieces usually have a water activity of about 0.5 to about 0.7.

The expanded pieces intended for human foods have a crispy, pleasant texture and good organoleptic properties. The flaked pieces also have good texture and organoleptic properties. The pieces have a pleasant taste of toasted cereal. Conveniently, the density of the pieces may be less than about 300 g/l. At this point, the expanded or flaked pieces usually have a water activity of about 0.15 to about 0.3.

The probiotic micro-organisms are then mixed into a suitable carrier substrate. The carrier substrate will vary depending on whether the pieces are intended for animals or humans. For pet foods, suitable carrier substrates include animal fats such as tallow, vegetable fats such as hydrogenated soy fat, protein digests which are commonly used as flavour coatings, and water. For human foods, suitable carrier substrates include liquids, such as fats and sugar solutions, and particulate coatings such as particulate flavour coatings. Suitable fats are edible vegetable oils and fats; for example hydrogenated soy fat. Suitable particulate flavour coatings include sugars, chocolate powders, milk powders, malted powders, flavoured beverage powders, and the like. If desired, the probiotic micro-organisms may be encapsulated.

Protection agents to improve the survival of the micro-organisms may be incorporated into the carrier substrate. Examples of suitable protecting agents are vitamins such as vitamins C and E, amino acids and their salts such as lysine, glycine, cysteine and sodium glutamate, sugars such as lactose, trehalose, saccharose, dextrine and maltodextrine, and proteins such as milk and soya proteins. Trace elements and minerals may also be included in the carrier substrate.

The selection of the carrier substrate will depend upon factors such as palatability considerations and the survival of the probiotic micro-organism since some micro-organisms survive better in some carrier substrates than others. For example, it is found that *S. cereviseae* may be slightly less stable in protein digests than in fats. If fats are used in the carrier substrate, the carrier substrate preferably contains antioxidants to reduce the action of oxygen on sensitive micro-organisms. However selecting the optimum carrier substrate is a matter of simple trial and error for the skilled person. If necessary, the carrier substrate may be heated slightly to melt it or to reduce its viscosity.

To produce a coated cereal product, any technique suitable for coating the pieces may be used. For example, in the case of a liquid carrier substrate, the mixture of the probiotic micro-organism and the carrier substrate may be sprayed onto the dried pieces. This may be carried out in any suitable manner. For example, the pieces may be fed into a fluidised bed onto which the mixture is sprayed. Alternatively, the pieces may be fed into a rotary coater into which the mixture is sprayed. As a further alternative, the pieces may be caused to fall in a curtain and the coating mixture sprayed onto the curtain. In the case of a particulate carrier substrate, the probiotic micro-organism and the carrier substrate may be mixed to form a dry mix. Heat sensitive components such as vitamins, amino acids, etc may also be included in the dry mix. The dry mix is then agglomerated on the dried pieces using an agglomerating agent. A suitable procedure is described in US patent 4,777,056; the disclosure of which is incorporated by reference. Fats, oils and sugar solutions are examples of suitable agglomerating agents. Particulate carrier substrates may also be dusted onto the cereal product.

For a filled cereal product, the mixture of the probiotic and micro-organism and carrier substrate is filled into the central bore of each piece. In this case, the carrier substrate is preferably viscous or a substance which hardens rapidly. Fats are particularly suitable. Alternatively the cereal product and carrier substrate may be fed into a tumbler and the carrier substrate agglomerated to the cereal product using a syrup. In this case, the cereal product is coated and filled.

The dried, ready-to-eat cereal product conveniently contains about 10^4 to about 10^{10} cells of the probiotic micro-organism per gram of the dried cereal product; preferably about 10^6 to about 10^8 cells of the probiotic micro-organism per gram. The dried cereal product may contain about 0.5% to about 20% by weight of the mixture of the probiotic micro-organism and carrier substrate; preferably about 1% to about 6% by weight; for example about 3% to about 6% by weight.

The dried cereal product may then be further processed as desired. For example, if the dried cereal is to be used as a breakfast cereal, dried fruit, nuts, other cereals, dried milk produce (such as dried yoghurt etc) may be dry mixed with or agglomerated with the coated cereal. If desired, the dried cereal may be further coated with protective agents or flavouring agents, or both. This may also be carried out prior to or during coating or filling of the dried pieces with the mixture of the probiotic and micro-organism and carrier substrate.

It is also possible to produce a dried cereal product by mixing together water and the ingredients of cereal product; for example in a preconditioner. The wet mixture may then be shaped into a desired shape; for example using shaping rollers. The shaped mixture may then be baked in an oven; for example at about 220°C to about 280°C for about 10 minutes to about 1 hour. The dried cereal product has the appearance of a baked biscuit. The coating or filling may then be applied as discussed above.

Alternatively the cereal product may be formulated into convenience foods such as snack bars and the like. Again the cereal product may be mixed with nuts, dried fruit, sugars or other sweeteners, colouring agents, or flavouring agents, and the like. A suitable binder, for example arabic gum or gelatine, may then be added. An agent which reduces breakability of the bar may also be included; for example hydrolysed wheat. If desired, the bar may be coated with a suitable coating; for example chocolate. Processes for manufacturing snack bars are well known and are described in the art; see for example US patent 4,871,557.

It will be appreciated that the dried, ready-to-eat cereal product may be produced by any suitable process and not only that described above.

In the case of human foods, the dried, ready-to-eat cereal product preferably comprises a nutritional supplement. In the case of pet foods, the dried, , ready-to-eat cereal product may be fed to pets as a sole source of nutrition or may be supplemented by other sources of nutrition; for example canned food. When consumed in adequate amounts, the dried, ready-to-eat cereal product results in a production of acids, such as lactic acid and acetic acid, in the gut of the human or animal. This inhibits the growth of pathogenic bacteria such as *Clostridium perfringens* or those which adversely affect well being, and has a beneficial effect on the human or animal. Also, the probiotic micro-organisms adhere to intestinal surfaces and compete with undesired bacteria. Further, the growth and activity of putrefying bacteria may be inhibited and hence the production of toxic amine compounds. Adequate amounts of the dried, ready-to-eat cereal product may also result in the activation of the immune function of the human or animal.

The amount of the dried, ready-to-eat cereal product to be consumed by the human or animal to obtain a beneficial effect will depend upon the size and age of the human or animal. However an amount of the dried, ready-to-eat cereal product to provide a daily amount of about 10^6 to about 10^{12} cells of the probiotic micro-organism would usually be adequate.

Numerous modifications may be made to the embodiments described above. For example, it is not necessary to

produce the cereal product by extrusion cooking. Instead the cereal product may be produced by any suitable method of producing dried, ready-to-eat cereal pieces. For example, the feed materials may be cooked with water to provided a cooked paste. The paste is then roller-dried to produce dried flakes; usually of a thickness of about 0.6 to about 1 mm.

Specific examples are now described for further illustration.

Example 1

A feed mixture is made up of corn, corn gluten, chicken and fish meal, salts, vitamins and minerals. The feed mixture is fed into a preconditioner and moistened. The moistened feed leaving the preconditioner is then fed into an extruder-cooker and gelatinised. The gelatinised matrix leaving the extruder is forced through a die and extruded. The extrudate leaving the die head is cut into pieces suitable for feeding to dogs, dried at about 110°C for about 20 minutes, and cooled to form pellets. The water activity of the pellets is about 0.6.

The pellets sprayed with three different coating mixtures. Each coating mixture contains *Bacillus coagulans* but one coating mixture uses hydrogenated soy fat as a coating substrate, one coating mixture uses water as a coating substrate, and one coating mixture uses a protein digest as a coating substrate. The *B. coagulans* is in the form of powdered endospores and is obtainable from Sankyo Pharmaceutical Company under the trade name Lacris-S. The pellets contain about 1.6 x 10^6 cells/g of *B. coagulans*. For each coating mixture, the pellets are separated into two groups. One group is stored at about 25°C and, to estimate the long term stability of the micro-organism, the other group is stored at about 37°C. A sample is taken of each group is taken after 1 week, 2 weeks, 3 weeks and 4 weeks. Also, a fat coated sample of the group which is stored at 37°C is taken at 8 weeks.

The cell counts are determined for each sample. The results are set out in Figure 1. In all cases, the cell counts remain substantially constant indicating excellent storage stability. Further, the results from the storage at 37°C for 8 weeks indicate that the micro-organisms are likely to be stable after one year of storage at normal conditions.

Example 2

Example 1 is repeated except that the three different coating mixtures each contain *Bacillus subtilis* instead of *Bacillus coagulans*. The *B. subtilis* is in the form of powdered endospores and is obtainable from Hansen A/S under the trade name BioPlus 2B. The results are set out in Figure 2.

In all cases, the cell counts remain substantially constant indicating excellent storage stability. However the cell counts for the pellets coated with fat are a little lower than those for water and protein digest but are still substantially constant. Again the results from the storage at 37°C for 8 weeks indicate that the micro-organisms are likely to be stable after one year of storage at normal conditions.

Example 3

Example 1 is repeated except that the three different coating mixtures each contain *Pediococcus acidilactici* instead of *Bacillus coagulans*. The *P. acidilactici* is in the form of a dried powder and is obtainable from Lallmand SA under the trade name Bactocell. The storage results are as follows:

Weeks	Fat 25°C	Fat 37°C	Water 25°C	Water 37°C	Digest 25°C	Digest 37°C
0	19.6x10^6	19.6 x10^6	21.9 x10^6	21.9 x10^6	12.9 x10^6	12.9 x10^6
1	13.6 x10^6	13.6 x10^6	14.7 x10^6	14.7 x10^6	12.1 x10^6	2.93 x10^6
2	12.9 x10^6	12.9 x10^6	13.1 x10^6	13.1 x10^6	-	-
3	9.73 x10^6	6.69 x10^6	16.0 x10^6	6.07 x10^6	7.77 x10^6	0.76 x10^6
4	12.9 x10^6	4.6 x10^6	14.0 x10^6	5.31 x10^6	-	-
5	-	-	-	-	5.1 x10^6	0.68 x10^6
8	6.8 x10^6	1.5 x10^6	-	-	-	

For the pellets coated using water or fats, the cell counts remain substantially constant at about 10^7 cfu/g; indicating excellent storage stability. For the pellets coated using protein digest, when stored at 37°C, the cell counts initially fall off but then stabilise at about 10^6 cfu/g; which is adequate.

Example 4

Example 1 is repeated except that the three different coating mixtures each contain *Saccharomyces cereviseae* instead of *Bacillus coagulans*. The *S. cereviseae* is in the form of a dried powder and is obtainable from Santel SA under the trade name Levucell. The storage results are as follows:

Weeks	Fat 25°C	Fat 37°C	Water 25°C	Water 37°C	Digest 25°C	Digest 37°C
0	28.0×10^6	28.0×10^6	27.6×10^6	27.6×10^6	11.3×10^6	11.3×10^6
1	23.2×10^6	23.3×10^6	17.2×10^6	17.2×10^6	7.53×10^6	1.83×10^6
2	24.5×10^6	24.5×10^6	18.7×10^6	18.7×10^6	-	-
3	24.5×10^6	9.93×10^6	13.5×10^6	4.40×10^6	1.99×10^6	0.16×10^6
4	13.7×10^6	15.9×10^6	-	-	-	-
5	-	-	-	-	2.42×10^6	0.03×10^6
8	17.5×10^6	12.3×10^6	-	-	-	

For the pellets coated using water or fats, the cell counts remain substantially constant at about 10^7 cfu/g; indicating excellent storage stability. This is particularly the case for the pellets coated with fats However the cell counts for the pellets coated with protein digest are a little lower than those for water and fat but are still acceptable when stored at 25°C. When stored at 37°C, the cell counts for the pellets coated with protein digest fall off.

Example 5

A trial is conducted using 30 dogs. The dogs are fed a standard dried diet for a week prior to commencement of the trials. Immediately prior to commencement of the trials, the gut flora and a measure of the faecal odours for each dog is determined.

The dogs are then separated into two groups of 15 dogs. One group of dogs is fed the dried, fat coated pellets of example 1. The other group of dogs is fed the same pellets but without the coating of fat and probiotic micro-organism. The dogs are given free access to the food and to water.

After a week, the gut flora of each dog is analysed. The dogs which are fed the pellets of example 1 have decreased counts of *C. perfringens*. Further, faecal pH and odours are found to have decreased in the dogs which are fed the pellets of example 1.

Example 6

A feed mixture is made up of 70% by weight of corn flour, 17% by weight of wheat flour, 7% by weight sugar, 3% by weight of malt, 2% by weight of vegetable fats, and salt. The feed mixture is fed into a preconditioner and moistened. The moistened feed leaving the preconditioner is then fed into an extruder and gelatinised. The gelatinised matrix leaving the extruder is forced through a die and extruded. The extrudate expands upon leaving the die head and is cut into pieces of about 1 cm. The pieces are then dried to a moisture content of about 1% by weight.

The pieces are sprayed with two different coating mixtures. Each coating mixture contains sunflower oil as the carrier substrate but a different micro-organism. The micro-organisms are:

Micro-organism	Source	Form
Saccharomyces cereviseae	Santel SA (Levucell™)	Spray dried powder
Enterococcus faecium SF68	Bioferment Division of Cerbios Pharma SA (LBC-K™), Switzerland	Spray dried powder

Both micro-organisms are commercially available. The pieces all contain about 10^6 cells/g to 10^7 cells/g of the pro-

biotic micro-organism. To obtain an idea of the long term stability of the micro-organism, the pieces are stored at about 37°C. A sample of each group is taken immediately after production, after 1 week and 3 weeks.

The viable cell counts are determined for each sample. The results are as follows:

Micro-organism	Cell count - Day 1 (cells/g)	Cell count - 1 week (cells/g)	Cell count - 3 weeks (cells/g)
Saccharomyces cereviseae	6.40×10^6	2.21×10^6	3.90×10^6
Enterococcus faecium SF68	1.38×10^6	8.60×10^6	4.03×10^6

The results indicate that the probiotic micro-organisms remain substantially stable.

Example 7

The procedure of example 6 is repeated except that the coating mixture is a dry mix of the probiotic micro-organisms and chocolate-flavoured powder (Nesquik® powder). The dry mix is coated on the pieces using the procedure described in US patent 4,777,056 and using vegetable oil as an agglomerating agent.

Further, the following micro-organisms are used:

Micro-organism	Source	Form
B. coagulans	Sankyo Pharmaceutical Company (Lacris-S™), Japan	Powdered endospores
L. johnsonii La1	Nestec SA	Freeze dried powder
Bifidobacterium animalis/longum	Ch. Hansen A/S (Bb12™), Denmark	Freeze dried powder
Saccharomyces cereviseae	Santel SA (Levucell™)	Spray dried powder
Enterococcus faecium SF68	Bioferment Division of Cerbios Pharma SA (LBC-K™), Switzerland	Spray dried powder

The first, third, fourth and fifth micro-organisms are commercially available. The second micro-organism is described in EP 0577904 and was deposited at the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, 28 rue du Dr Roux, 757724 Paris Cedex 15, France on 30 June 1992 under the number CNCM I-1225 and name La 1 by Nestec S.A.

The cell counts determined for each sample. The results are as follows:

Micro-organism	Cell count - Day 1 (cells/g)	Cell count - 1 week (cells/g)	Cell count - 3 weeks (cells/g)
B. coagulans	6.37×10^6	5.07×10^6	4.24×10^6
L.johnsonii La1	1.43×10^6	3.21×10^5	1.39×10^5
Bifidobacterium animalis/longum	8.06×10^6	2.95×10^6	9.80×10^5
Saccharomyces cereviseae	2.43×10^5	2.17×10^5	1.38×10^5
Enterococcus faecium SF68	1.94×10^6	5.70×10^5	1.50×10^4

The results indicate that the *B. coagulans* and *Bifidobacterium animalis/longum* are likely to remain stable for long periods. The other micro-organisms display less but acceptable stability.

Example 8

Expanded cereal products produced as described in example 6 are coated with three coating substrates. Product 1 is prepared by coating the cereal product with vegetable oil and then dusting on a spray-dried milk powder which contains *L. johnsonii* La1; Product 2 is prepared by coating the cereal product with vegetable oil and then dusting on a mixture of a spray-dried milk powder which contains *L. johnsonii* La1 and a cocoa-containing powder (Nesquik® powder); Product 3 is prepared by suspending a spray-dried milk powder which contains *L. johnsonii* La1 in a vegetable oil and spraying the oil (without pressure) on the cereal product.

The cell counts determined for each product. The results are as follows:

Product	Cell count - Day 1 (cells/g)	Cell count - 1 week (cells/g)	Cell count - 3 weeks (cells/g)
1	3.86×10^7	4.42×10^7	3.00×10^7
2	1.59×10^7	2.30×10^7	1.65×10^7
3	3.51×10^7	4.61×10^6	3.36×10^5

The results indicate that the probiotic micro-organisms remain substantially stable.

Example 9

A trial is conducted using 20 adult volunteers. Immediately prior to commencement of the trials, the gut flora of each volunteer is determined. The volunteers are then separated into two groups of 10 people. One group is fed, for breakfast, a 30 g portion of product 1 of example 8 along with cold milk. The other group is fed the same cereal product but without the coating of fat and probiotic micro-organism. Other meals during the day are the normal meals eaten by the volunteers.

After a week, the gut flora of each volunteer is analysed. The volunteer which are fed Product 1 have decreased counts of *C. perfringens*.

Claims

1. A dried, ready-to-eat cereal product comprising a gelatinised starch matrix which includes a coating or filling containing a probiotic micro-organism.

2. A cereal product according to claim 1 in the form of a breakfast cereal, an infant cereal, or a convenience food.

3. A cereal product according to claim 1 or claim 2 in which the gelatinised starch matrix is in flaked or expanded form.

4. A cereal product according to claim 1 in the form of a pet food.

5. A cereal product according to any of claims 1 to 4 in which the gelatinised matrix is an extrusion cooked starch source.

6. A cereal product according to any of claims 1 to 5 in which the coating or filling comprises a carrier substrate which contains the probiotic micro-organism.

7. A cereal product according to claim 6 in which the carrier substrate is a fat, a protein digest, milk solids, a sugar or a particulate flavouring agent.

8. A cereal product according to claim 5 further comprising a lipid layer on the gelatinised starch matrix, the lipid layer causing a particulate carrier substrate, which contains the probiotic micro-organism, to adhere to the gelatinised starch matrix.

9. A cereal product according to any of claims 1 to 8 in which the probiotic micro-organism is selected from *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bifidobacterium animalis/longum*, *L. johnsonii* La1, *Pediococ-*

cus acidilactici, *Saccharomyces cereviseae*, and *Enterococcus faecium* SF68.

10. A cereal product according to any one of claims 1 to 9 further containing a source of soluble fibre.

FIG.1.

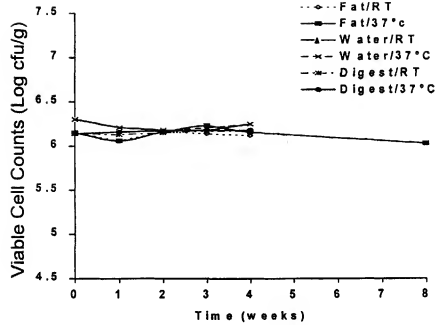
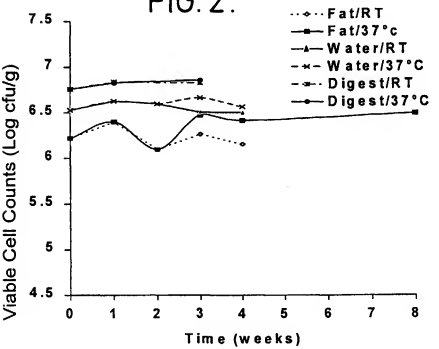

Fat/RT
Fat/37°c
Water/RT
Water/37°C
Digest/RT
Digest/37°C
Viable Cell Counts (Log cfu/g)
7.5
7
6.5
6
5.5
5
4.5
0
1
2
3
4
5
6
7
8
Time (weeks)

FIG. 2.


Fat/RT
Fat/37°c
Water/RT
Water/37°C
Digest/RT
Digest/37°C
Viable Cell Counts (Log cfu/g)
7.5
7
6.5
6
5.5
5
4.5
0
1
2
3
4
5
6
7
8
Time (weeks)